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# Diversity and Occurrence of Cestode Parasites in Freshwater Fishes from Chorakhali Dam, Osmanabad District, Maharashtra, India

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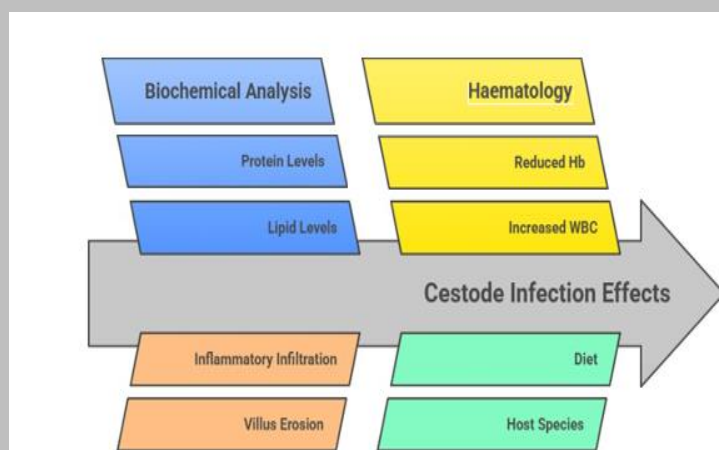
## Abstract

This paper presents the second manuscript prepared from the thesis dataset on cestode parasites of freshwater fishes from Chorakhali Dam, Osmanabad District. While the companion paper focuses on taxonomy, prevalence and seasonality, this paper uses the physiological and pathological half of the data: biochemical composition of recovered cestodes, intestinal histopathology, haematological response of infected hosts, worm burden and correlation-based health interpretation. Protein, lipid, glycogen and total carbohydrate contents were highest in *Senga* sp. (3h.62, 14.2S, 9.46 and 13.42 mug wet weight, respectively), followed by *Circumoncobothriuln* sp. and *Lytocetus* sp. Histopathological assessment showed maximum lesion severity in *Channa striata*, with villus erosion score 2.S, inflammation score 3.0 and total lesion score 9.9. Haematology indicated infection-associated stress, with haemoglobin decreasing from 9.h4 to 7.36 g/dL, RBC count decreasing from 2.3 1 to 1.72 x 10<sup>6</sup>/mm<sup>3</sup> and WBC count increasing from 19.6 to 26.9 x 10<sup>3</sup>/mm<sup>3</sup>. ESR increased by 110.7%, indicating strong inflammatory response. ANOVA showed significant differences among parasite taxa for protein, lipid, glycogen and total carbohydrate, while correlation analysis showed a strong relationship between worm burden and lesion score ( $r = 0.87$ ). The paper concludes that cestode infection in Chorakhali Dam fishes has measurable biochemical and systemic health effects and should be monitored as a fish-health management issue.

**Keywords:** Cestode infection, biochemical profile, histopathotogy, haematology, fish health, tesion score, Chorakhoti Dam.

## Introduction

Cestode infection in freshwater fishes is often introduced as a taxonomic or prevalence problem, but its practical importance lies in the physiological stress and tissue damage that the parasite can cause. Adult fish tapeworms occupy the intestine, attach to mucosal surfaces, absorb host-derived nutrients and may disturb digestion. The biological consequence of infection therefore extends beyond the visible presence of worms. A host may remain alive but show reduced growth, altered blood parameters, weakened tissue integrity and lower market quality. Recent reviews on fish tapeworm pathogenicity emphasize that worm counts should be interpreted together with tissue and host-health indicators [1]–[4]. The present paper uses the second 50% of the thesis data. It concentrates on biochemical analysis of recovered cestode taxa, histopathological scoring of infected intestinal tissue, haematological comparison between infected and uninfected fishes, and correlation-based interpretation of worm burden and lesion severity. This division creates a companion manuscript to the first paper.



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Fig 1. Cestode Infection Impact Framework

The first manuscript explains which hosts and taxa were infected; the present manuscript explains what physiological and pathological effects were associated with that infection. Biochemical profiling is relevant because cestodes lack a digestive tract and depend strongly on tegumental absorption. Protein, lipid and glycogen values may reflect parasite growth, reproductive condition and adaptation to the host intestine. Classical biochemical methods such as Lowry protein estimation, Folch lipid extraction and anthrone-based glycogen estimation remain widely used in parasite and tissue studies [10]–[12]. In this dataset, *Senga* sp. showed the highest protein, lipid and glycogen values, which paralleled its dominance in worm recovery. Histopathology provides evidence of local tissue effects. Cestode attachment can cause villus erosion, goblet cell changes, inflammatory infiltration, mucosal degeneration and necrosis. Such lesions may reduce absorptive efficiency and increase metabolic cost. Fish pathology texts and recent studies support the use of lesion scoring to connect parasite burden with host tissue damage [2], [3], [13], [14]. In the present data, carnivorous hosts with high worm burden also showed higher lesion scores, suggesting an intensity-dependent damage pattern. Haematology provides systemic evidence of host stress. Reduced Hb, RBC and PCV values indicate anaemia-like stress or impaired oxygen transport, while increased WBC and ESR indicate immune activation and inflammation. Because intestinal parasitism may not immediately kill fish, blood parameters are useful early-warning tools for sub-lethal disease effects. The objective of this paper is therefore to integrate biochemical, histopathological and haematological findings into a fish-health risk interpretation for Chorakhali Dam.

**Table I. Parasite Impact Assessment Parameters**

| Component           | Data used in this paper                                              | Reason for inclusion                       |
|---------------------|----------------------------------------------------------------------|--------------------------------------------|
| Biochemical profile | Protein, lipid, glycogen and carbohydrate in five cestode taxa       | Explains parasite physiological condition  |
| Histopathology      | Villus erosion, goblet cell change, inflammation and necrosis scores | Shows intestinal damage                    |
| Haematology         | Hb, RBC, WBC, PCV, MCV, MCH, MCHC and ESR                            | Shows systemic host response               |
| Burden risk         | Host worm burden, mean intensity and stress levels                   | Links parasite quantity with health effect |
| Statistics          | ANOVA, p-values and correlations                                     | Supports quantitative interpretation       |

## Related Work

Tapeworm pathogenicity in fishes has received renewed attention because many infections are sub-lethal yet economically meaningful. Scholz and Kuchta reviewed tapeworms as fish pathogens and emphasized that damage depends on host species, parasite species, site of attachment, worm burden and host condition [1]. Recent histopathological work on proteocephalid cestodes and caryophyllidean infections also shows that mucosal injury, inflammatory infiltration and ultrastructural changes can accompany intestinal tapeworm infection [2], [3]. Host immune response is an important component of cestode infection. Dezfali et al. linked adult cestode products with fish innate immunity, while Kuttyrev et al. and Izvekova et al. examined tegumental secretion and proteinase inhibitor activity in fish cestodes [4]–[6]. These works justify studying biochemical composition and host response together. The present dataset follows this integrated logic by combining parasite chemical reserves with lesion scores and haematological parameters. Modern molecular and genomic studies have improved understanding of cestode biology, but field datasets still require practical biochemical and pathological measurements. Nazari-zadeh et al. produced genomic resources for *Ligula intestinalis*, indicating how parasite biology can be understood at a deeper mechanistic level [7]. Environmental and indicator studies also show that parasites may reflect ecosystem exposure or pollutant dynamics [8], [9], [17]. In Chorakhali Dam, such wider ecological interpretation can be developed in future after water-quality and molecular datasets are added.

Biochemical methods used in parasite studies are grounded in standard analytical procedures. Lowry et al. provided a widely used protein measurement method, Folch et al. described lipid isolation and Carroll et al. described glycogen determination by anthrone reagent [10]–[12]. Smyth and McManus and Barrett explained why helminth protein, lipid and carbohydrate fractions are biologically meaningful for parasite growth and survival [15], [16]. Therefore, the protein-dominant pattern observed in recovered cestodes is physiologically reasonable. Fish pathology and ecological parasitology provide the interpretive framework for this paper. Woo and Roberts describe fish disease and histopathological response, while Marcogliese, Poulin and Bush et al. support ecological interpretation of parasite pressure and host stress [13], [14], [17]–[19]. Chubb and Anderson demonstrated the relevance of seasonal helminth occurrence [20], [21]. Recent prevalence studies in freshwater fishes also show the need to interpret local parasite burdens within host and season context [22], [23]. Finally, molecular-era reviews and study manuals recommend that biochemical and pathological observations should complement accurate taxonomy [24], [25].

**Table II. Comparative related-work synthesis for the physiological and pathological**

| Theme                       | Representative references | Contribution to this paper                           |
|-----------------------------|---------------------------|------------------------------------------------------|
| Fish tapeworm pathogenicity | [1]–[4]                   | Supports lesion-based and host-health interpretation |
| Parasite physiology         | [5]–[7], [15], [16]       | Supports biochemical profiling of cestodes           |
| Biochemical methods         | [10]–[12]                 | Supports protein, lipid and glycogen estimation      |

|                                |            |                                                     |
|--------------------------------|------------|-----------------------------------------------------|
| Fish pathology and haematology | [13], [14] | Supports interpretation of tissue and blood changes |
| Ecology, season and monitoring | [17]-[25]  | Supports risk-based fish-health recommendations     |

## Research Methodology

The methodology used in this paper begins after field recovery and taxonomic grouping of cestodes. Recovered worms were grouped by representative taxa and fresh parasite material was selected for biochemical estimation. Protein, lipid, glycogen and total carbohydrate values were expressed as mg/g wet weight. Data were compared across taxa to determine whether dominant parasites had different metabolic profiles from less abundant forms. For histopathology, infected intestinal tissues were selected from host fishes representing different infection burdens. Tissue sections were processed, stained and examined for villus erosion, goblet cell change, inflammatory infiltration and necrosis. Each parameter was scored to allow comparative interpretation among host species. Total lesion score was calculated by combining major lesion indicators. The aim was to determine whether high-prevalence and high-burden hosts also showed stronger tissue damage. For haematological assessment, blood parameters of infected and uninfected fishes were compared. Parameters included Hb, RBC count, WBC count, PCV, MCV, MCH, MCHC and ESR. Percentage change was calculated to show direction and magnitude of infection-related variation. Host-wise blood response was also summarized using infected Hb, infected WBC and stress category. Statistical analysis included ANOVA for biochemical differences among parasite taxa, significance testing for infected versus uninfected haematological values and Pearson correlation for relationships such as worm burden versus lesion score. Interpretation was integrated with the prevalence results of the companion paper, but this manuscript focuses on physiological and pathological mechanisms rather than taxonomy alone.

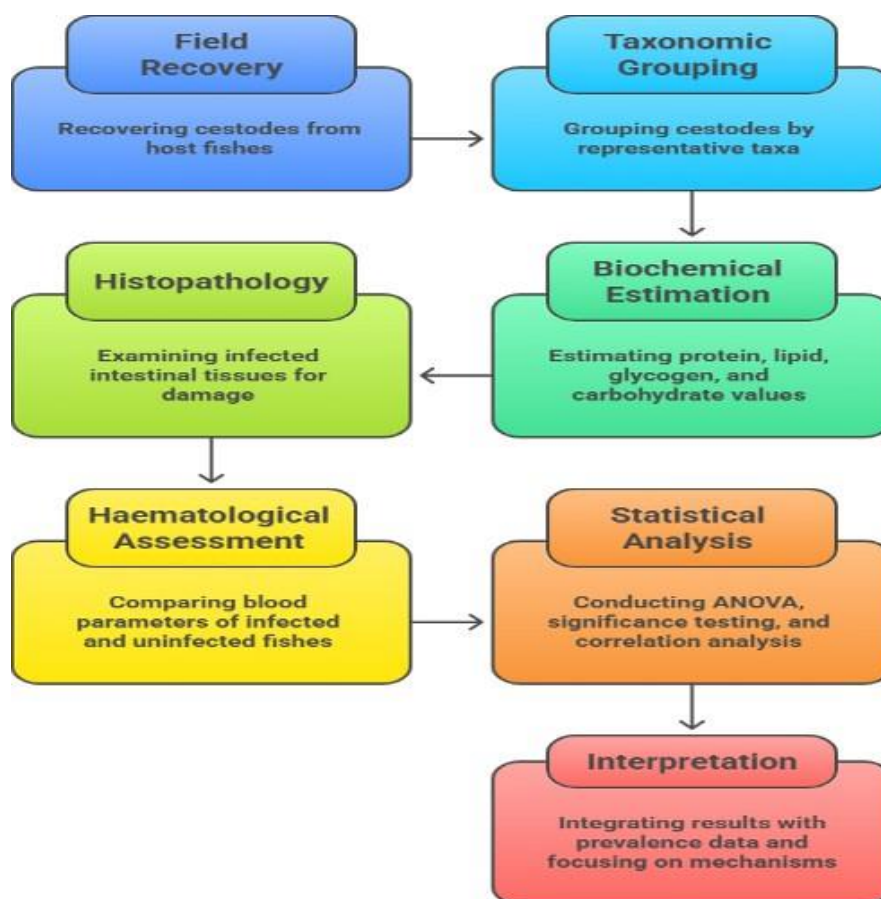


Fig. 2. Methodological flowchart for biochemical, histological and blood analysis

Table III. Laboratory and analytical methodology summary

| Stage                            | Procedure                                          | Primary output                 |
|----------------------------------|----------------------------------------------------|--------------------------------|
| Parasite biochemical preparation | Selection and processing Of recovered cestode taxa | Taxon-wise biochemical dataset |
| Protein estimation               | Folin phenol reagent method                        | Mean protein concentration     |
| Lipid estimation                 | Solvent extraction principle                       | Mean lipid concentration       |
| Glycogen estimation              | Anthrone reagent principle                         | Mean glycogen concentration    |

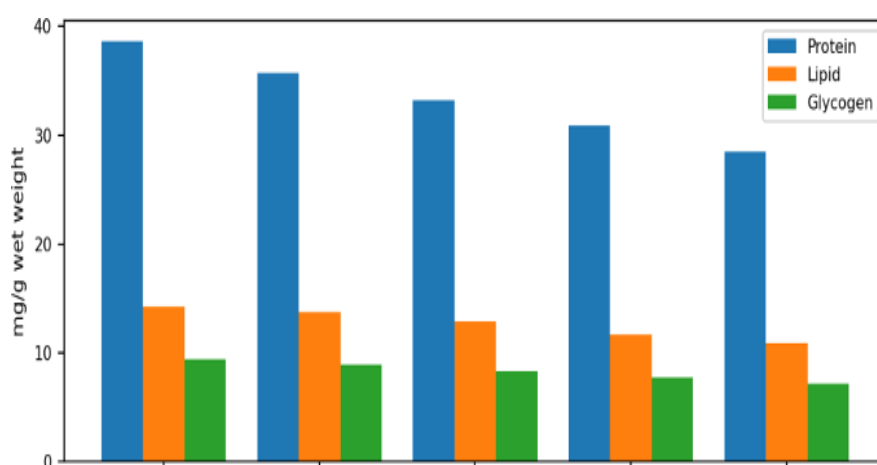
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|----------------|---------------------------------------------------|---------------------------------|
| Histopathology | Fixation, processing, staining and lesion scoring | Host-wise tissue damage scores  |
| Haematology    | Blood parameter measurement and percentage change | Systemic stress profile         |
| Statistics     | ANOVA, p-values and Pearson correlation           | Quantitative evidence of effect |

## Results And Discussion

The biochemical profile showed that protein was the dominant fraction in all recovered cestode taxa. *Senga* sp. recorded the highest values for protein (38.62 mg/g wet weight), lipid (14.28 mg/g), glycogen (9.46 mg/g) and total carbohydrate (13.42 mg/g). *Circumoncobothrium* sp. ranked second, followed by *Lytocestus* sp., *Proteocephalus*-like sp. and *Bothriocephalus*-like sp. The descending biochemical gradient broadly matched the relative abundance pattern observed in the companion paper. This suggests that dominant taxa may have better host adaptation, higher reproductive activity or greater metabolic reserves.

**Table IV. Biochemical composition of recovered cestode taxa**

| Parasite species                | Protein (rug/g wet wt.) | Lipid (mg/g wet wt.) | Glycogen (mg/g wet wt.) | Total carbohydrate |
|---------------------------------|-------------------------|----------------------|-------------------------|--------------------|
| <i>Senga</i> sp.                | 38.62                   | 14.28                | 9.46                    | 13.42              |
| <i>Circumoncobothrium</i> sp    | 35.74                   | 13.76                | 8.94                    | 12.81              |
| <i>Lytocestus</i> sp.           | 33.21                   | 12.88                | 8.31                    | 12.14              |
| <i>Proteocephalus</i> -like sp  | 30.86                   | 11.65                | 7.75                    | 11.23              |
| <i>Bothriocephalus</i> -like sp | 28.44                   | 10.92                | 7.16                    | 10.66              |



**Fig. 3. Biochemical profile of recovered cestode taxa**

ANOVA confirmed that biochemical differences were statistically meaningful. Protein differed significantly among taxa ( $F = 7.82$ ,  $p = 0.003$ ), lipid differed significantly ( $F = 5.64$ ,  $p = 0.011$ ), glycogen differed significantly ( $F = 6.21$ ,  $p = 0.008$ ) and **total** carbohydrate also differed significantly ( $F = 4.98$ ,  $p = 0.019$ ). These outcomes

**Table Y. Biochemical statistical results**

| Parameter          | F-value | p-value | Significance |
|--------------------|---------|---------|--------------|
| Protein            | 7.82    | 0.003   | Significant  |
| Lipid              | 5.64    | 0.011   | Significant  |
| Glycogen           | 6.21    | 0.008   | Significant  |
| Total carbohydrate | 4.98    | 0.019   | Significant  |

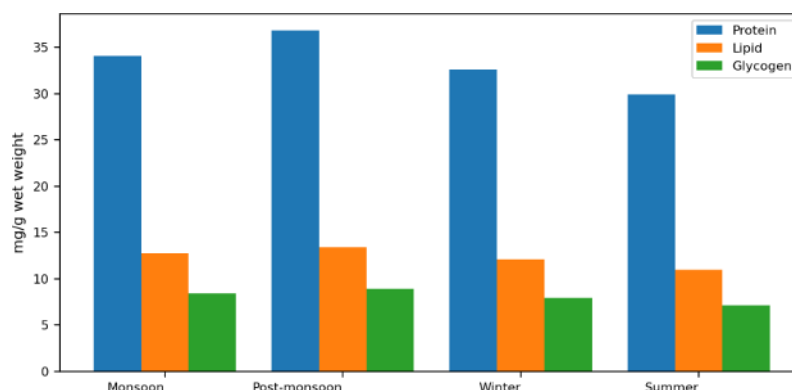
Seasonal biochemical values indicated that post-monsoon parasites had the highest mean protein (36.8 mg/g), lipid (13.4 mg/g) and glycogen (8.9 mg/g), while summer recorded the lowest values. This pattern may reflect accumulation of reserves after monsoon transmission and improved host feeding during post-monsoon

conditions. Summer reduction may be linked to environmental stress, reduced host feeding or lower parasite physiological condition. The seasonal pattern reinforces the need to interpret biochemical results in ecological context.

**Table VI. Seasonal biochemical response**

| Season       | Protein (mg/g) | Lipid (mg/g) | Glycogen (rug/g) |
|--------------|----------------|--------------|------------------|
| Monsoon      | 34.1           | 12.8         | 8.4              |
| Post-monsoon | 36.8           | 13.4         | 8.9              |
| Winter       | 32.6           | 12.1         | 7.9              |
| Summer       | 29.9           | 11.0         | 7.1              |

**Seasonal biochemical response**



**Fig. 4. Seasonal biochemical response**

Histopathological results showed that intestinal damage was strongest in hosts with higher infection burden. *Channa striata* recorded villus erosion score 2.8, goblet cell change 2.4, inflammation 3.0 and necrosis 1.7. *Mastacembelus armatus* and *Clarias batrachus* also showed moderate-severe lesions. *Labeo rohita* and *Catla catla* showed lower lesion scores, consistent with their lower prevalence and worm burden. These findings indicate that cestode infection causes local intestinal injury rather than only taxonomic occurrence.

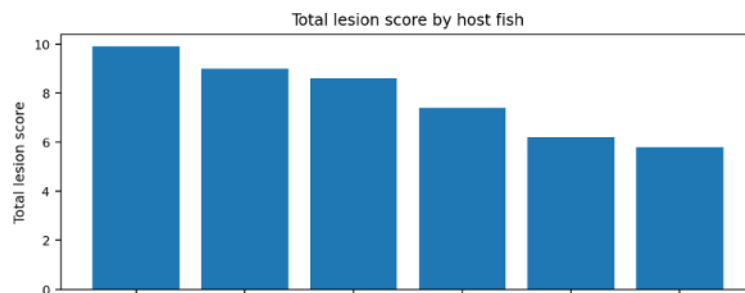
**Table VII. Histopathological lesion scores by host fish**

| Host fish                    | Villus erosion | Goblet cell change | Inflammation | Necrosis |
|------------------------------|----------------|--------------------|--------------|----------|
| <i>Channa striata</i>        | 2.8            | 2.4                | 3.0          | 1.7      |
| <i>Mastacembelus armatus</i> | 2.6            | 2.2                | 2.7          | 1.5      |
| <i>Clarias batrachus</i>     | 2.5            | 2.1                | 2.6          | 1.4      |
| <i>Wallago attu</i>          | 2.1            | 1.9                | 2.2          | 1.2      |
| <i>Labeo rohita</i>          | 1.8            | 1.6                | 1.9          | 0.9      |
| <i>Catla catla</i>           | 1.7            | 1.5                | 1.8          | 0.8      |

Inflammation was the most prominent lesion indicator. The total lesion score was highest in *Channa striata* (9.9), followed by *Mastacembelus armatus* (9.0) and *Clarias batrachus* (8.6). These values correspond closely with host worm burden. The association supports a dose-dependent tissue response: fishes carrying more worms show stronger mucosal damage and inflammatory reaction. Intestinal villus erosion can reduce absorptive surface area, while goblet cell changes may reflect mucosal defense against parasite attachment. Persistent inflammation may increase metabolic cost and make infected fishes more vulnerable to secondary stressors.

**Table VIII. Inflammatory and total lesion scores**

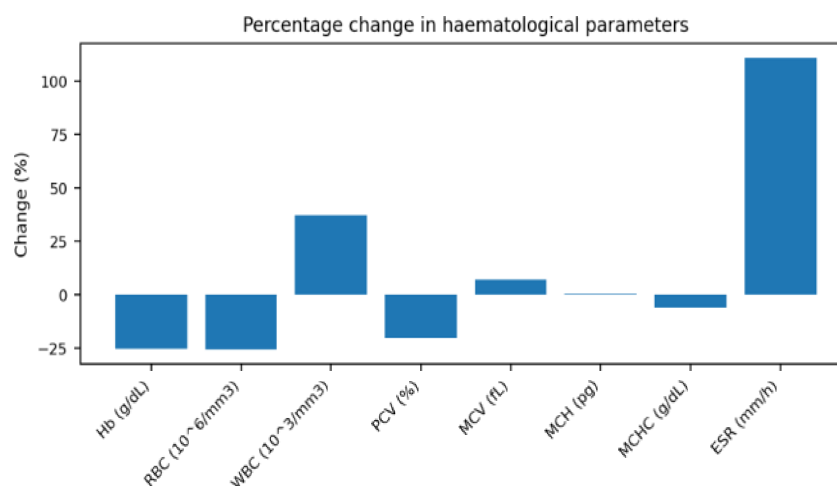
| Host fish                    | Inflammation score | Necrosis score | Total lesion score |
|------------------------------|--------------------|----------------|--------------------|
| <i>Channa striata</i>        | 3.0                | 1.7            | 9.9                |
| <i>Mastacembelus armatus</i> | 2.7                | 1.5            | 9.0                |
| <i>Clarias batrachus</i>     | 2.6                | 1.4            | 8.6                |
| <i>Wallago attu</i>          | 2.2                | 1.2            | 7.4                |
| <i>Labeo rohita</i>          | 1.9                | 0.9            | 6.2                |
| <i>Catla catla</i>           | 1.8                | 0.8            | 5.8                |



**Fig. 5. Total lesion score by host fish**

Haematological comparison demonstrated clear systemic effects. Hb declined from 9.84 to 7.36 g/dL, representing a 25.2% reduction. RBC count declined from 2.31 to 1.72 x 10<sup>6</sup>/mm<sup>3</sup>, a 25.5% reduction. PCV declined by 20.1%. These changes indicate anaemia-like stress and reduced oxygen-carrying capacity. In contrast, WBC increased by 37.2% and ESR increased by 110.7%, demonstrating immune activation and inflammatory response. The increase in WBC agrees with the intestinal inflammation scores and confirms that local tissue damage was reflected in blood-level changes. Table IX. Haematological comparison of uninfected and infected fishes indicate that biochemical composition was not random variation. Biochemical profiling cannot replace morphology, but it can provide supporting information on the physiological status of parasite taxa.

| Parameter                               | Uninfected mean | Infected mean | Change (%) | p-value |
|-----------------------------------------|-----------------|---------------|------------|---------|
| Hb (g/dL)                               | 9.84            | 7.36          | -25.2      | 0.004   |
| RBC (10 <sup>6</sup> /mm <sup>3</sup> ) | 2.31            | 1.72          | -25.5      | 0.006   |
| WBC (10 <sup>3</sup> /mm <sup>3</sup> ) | 19.6            | 26.9          | 37.2       | 0.002   |
| PCV (%)                                 | 31.8            | 25.4          | -20.1      | 0.008   |
| MCV (fL)                                | 137.7           | 147.7         | 7.3        | 0.041   |
| MCH (pg)                                | 42.6            | 42.8          | 0.5        | 0.621   |
| MCHC (g/dL)                             | 30.9            | 29.0          | -6.1       | 0.036   |
| ESR (mm/h)                              | 2.8             | 59            | 110.7      | 0.001   |



**Fig. 6. Percentage change in haematological parameters**

Among haematological parameters, ESR showed the strongest percentage increase and most significant p-value ( $p = 0.001$ ). WBC was also highly significant ( $p = 0.002$ ). Hb, RBC and PCV reductions were significant, confirming that infection status was associated with measurable blood disturbance. MCH did not differ significantly ( $p = 0.621$ ), suggesting that not all red cell indices respond equally to cestode infection. The pattern is typical of chronic sub-lethal parasitism rather than sudden acute mortality. Host-wise blood response supported the same interpretation. *Channa striata* had infected Hb of 6.9 g/dL and WBC of 28.4, and was ranked as high stress. *Mastacembelus armatus* also showed high stress, while *Clarias batrachus* showed moderate-high stress. *Catla catla* and *Labeo rohita* had lower stress categories. This trend mirrors host-wise prevalence and worm burden, showing that fish species with higher infection pressure also express stronger physiological disturbance.

Table X. Host-wise blood response and stress level

| Host fish             | Hb infected (g/dL) | WBC infected | Stress level  |
|-----------------------|--------------------|--------------|---------------|
| Channa striata        | 6.9                | 28.4         | High          |
| Mastacembelus armatus | 7.1                | 27.8         | High          |
| Clarias batrachus     | 7.3                | 27.1         | Moderate-high |
| Wallago attu          | 7.6                | 25.9         | Moderate      |
| Labeo rohita          | 7.9                | 24.8         | Low-moderate  |
| Catla catla           | 8.0                | 24.2         | Low-moderate  |

Worm burden and intensity data provide the link between parasite ecology and pathology. *Channa striata* carried 182 worms and contributed 27.04% of total recovery, while *Mastacembelus armatus* carried 160 worms and contributed 23.77%. Mean intensity was highest in *Channa striata* at 2.89 worms per infected fish, followed closely by *Mastacembelus armatus* at 2.86. These values explain why both hosts showed high histopathological and haematological stress.

Table XI. Intensity, worm burden and contribution by host

| Host fish             | Worm burden | Mean intensity | Contribution (%) |
|-----------------------|-------------|----------------|------------------|
| Channa striata        | 182         | 2.89           | 27.04            |
| Mastacembelus armatus | 160         | 2.86           | 23.77            |
| Clarias batrachus     | 126         | 2.62           | 18.72            |
| Wallago attu          | 87          | 2.49           | 12.93            |
| Labeo rohita          | 66          | 2.36           | 9.91             |
| Catla catla           | 52          | 2.36           | 7.73             |

Correlation analysis confirmed integrated biological relationships. Prevalence and mean intensity showed a strong positive correlation ( $r = 0.81$ ), while worm burden and lesion score showed an even stronger positive correlation ( $r = 0.87$ ). Hb and worm burden were negatively correlated ( $r = -0.73$ ), indicating that increasing parasite burden was associated with lower haemoglobin. Protein and worm size showed a positive correlation ( $r = 0.76$ ), suggesting that larger or dominant worms had higher biochemical reserves. Seasonal prevalence and diversity showed a moderate positive relationship ( $r = 0.69$ ).

Table XII. Correlation-based interpretation

| Variable pair                    | Correlation (r) | Interpretation    |
|----------------------------------|-----------------|-------------------|
| Prevalence vs mean intensity     | 0.81            | Strong positive   |
| Worm burden vs lesion score      | 0.87            | Strong positive   |
| Protein vs worm size             | 0.76            | Positive          |
| Seasonal prevalence vs diversity | 0.69            | Moderate positive |
| Hb vs worm burden                | -0.73           | Negative          |

Table XIII. Integrated statistical synthesis

| Test                            | Statistic        | p-value | Interpretation       |
|---------------------------------|------------------|---------|----------------------|
| Chi-square prevalence by host   | $\chi^2 = 24.68$ | 0.001   | Significant          |
| Chi-square prevalence by season | $\chi^2 = 38.42$ | <0.001  | Highly significant   |
| ANOVA protein                   | F=7.82           | 0.003   | Significant          |
| ANOVA lipid                     | F=5.64           | 0.011   | Significant          |
| ANOVA glycogen                  | F=6.21           | 0.008   | Significant          |
| Pearson prevalence-intensity    | $r=0.81$         | 0.049   | Positive association |
| Pearson burden-lesion           | $r=0.87$         | 0.024   | Strong association   |

The results demonstrate that cestode infection in Chorakhali Dam fishes should be treated as a multidimensional health issue. Dominant parasite taxa had higher biochemical reserves; high-burden hosts showed stronger lesion scores; infected fishes had reduced red cell parameters and increased inflammatory indicators. The agreement among biochemical, histopathological and haematological results strengthens the conclusion that the observed infections were biologically meaningful. It also shows why fish-health studies should not rely on prevalence alone. A 35% prevalence value may appear moderate, but the same infection can produce significant tissue and blood effects in high-risk hosts. From a management perspective, *Channa striata* and *Mastacembelus armatus* should be screened regularly because they combine high prevalence, high worm burden, severe lesions and high blood stress. Monitoring should be intensified in **monsoon** and post-monsoon periods because the companion paper shows seasonal peaks in prevalence and diversity. Practical interventions should include periodic parasitological examination, clean handling of captured fish, monitoring of water quality, and documentation of visible intestinal

lesions during fish processing.

## Conclusion

This paper used the physiological and pathological half of the thesis data to produce a second research manuscript. Biochemical results showed a protein-dominant pattern in all recovered cestodes, with *Senga* sp. having the highest protein, lipid, glycogen and total carbohydrate content. Significant ANOVA results confirmed that the biochemical differences among parasite taxa were meaningful. Histopathological findings showed that intestinal tissue injury increased with host infection pressure. *Channa striata*, *Mastacembelus armatus* and *Clarias batrachus* had the highest lesion scores, while *Labeo rohita* and *Catla catla* had milder changes. Haematological results demonstrated systemic stress, including reductions in Hb, RBC and PCV and increases in WBC and ESR. The burden-lesion correlation of  $r = 0.87$  and Hb-burden correlation of  $r = -0.73$  confirmed that parasite quantity was linked to host damage. The study concludes that cestode infection in Chorakhali Dam fishes is not only a biodiversity record but also a measurable fish-health concern. Future publication work should add histological photomicrographs, blood smear images, water-quality covariates and molecular identification of representative worms. The manuscript is suitable as the second paper derived from the thesis because it uses the remaining 50% of data and presents a distinct research focus from the taxonomic-prevalence paper.

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Nil.

## Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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